Ascorbic Acid Status and Subsequent Diastolic and Systolic Blood Pressure

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Abstract—Free radicals and oxidation are involved in several aspects of blood pressure physiology. We investigated the relationship between blood pressure and antioxidants, including plasma ascorbic acid (AscA), in a 17-week controlled-diet study. Study subjects included 68 men aged 30 to 59 years who had a mean diastolic blood pressure of 73.4 mm Hg and a mean systolic blood pressure of 122.2 mm Hg. One month of vitamin C depletion was followed by 1-month repletion with 117 mg/d, repeated twice. All food and drink were provided in the study. Subjects did not smoke or drink alcohol, all consumed fruits and vegetables, and body weight was maintained. Plasma was assayed periodically for AscA, α-tocopherol, carotenoids, and lipids. Plasma AscA was inversely related to diastolic blood pressure 1 month later (correlation -0.48, $P<0.0001$). Persons in the bottom fourth of the plasma AscA distribution had >7 mm Hg higher diastolic blood pressure than did those in the top fourth of the plasma AscA distribution. Multivariate analysis with control for age, body mass index, other plasma antioxidants, and dietary energy, calcium, fiber, sodium, and potassium did not reduce the plasma AscA effect. One fourth of the variance in diastolic blood pressure was accounted for by plasma AscA alone. Plasma AscA was also significantly associated with systolic blood pressure in logistic regression. Vitamin C may be an important component of the effectiveness of fruits and vegetables in the reduction in blood pressure, and tissue AscA levels may be important in the maintenance of low blood pressure. Long-term intervention studies are warranted. **(*Hypertension*. 2001;37:261-267.)**

Key Words: blood pressure ■ nutrition ■ diet ■ ascorbic acid ■ antioxidants

Recent research has demonstrated a host of mechanisms by which antioxidants could affect blood pressure (BP). The Dietary Approaches to Stop Hypertension[1] (DASH) study demonstrated that a diet rich in fruits and vegetables can significantly reduce BP in persons with moderate BP elevation; fruits and vegetables are major sources of antioxidants, as well as other nutritional factors such as fiber and potassium. Although a varied diet may confer benefits greater than those of any single nutrient, it would be useful to determine whether a particular nutrient plays a major regulatory role. In the present study, in which diet and other factors were tightly controlled, we examined the role of vitamin C and several other antioxidants in influencing BP.

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A recent review of research on the relationship between vitamin C and BP[2] found an inverse association of vitamin C with BP to be "reasonably consistent across different studies and populations, with a variety of study designs." Several cross-sectional studies were found to have highly significant inverse relationships ($P<0.001$) between BP and plasma ascorbic acid (AscA). More recently, Bates et al[3] found ascorbate to be the only plasma nutrient negatively and significantly associated with BP. Finally, a recent case-comparison study found significantly lower AscA levels in hypertensives (40 μmol/L) than in normotensives (57 μmol/L).[4]

Cross-sectional studies, however, provide only weak evidence for a causal relationship, because vitamin C intake and blood levels are correlated with other factors, such as dietary fiber and carotenoids. A number of intervention studies have been conducted, but most have had various methodological problems that make them difficult to interpret.[2] Many were very small, and some had no control group, permitted placebo subjects to take multivitamins, did not exclude smokers, did not control alcohol or other dietary intake, or did not control body weight changes or other factors that might affect BP. A few intervention studies have been conducted since the review by Ness et al.[5–8] Miller et al[7] administered vitamin C, vitamin E, and beta-carotene to 297 older persons and found no BP difference between the antioxidant and placebo groups. However, all subjects, including the placebo group, were permitted to take a multivitamin that contained vitamins C and E and beta-carotene during the

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TABLE 1. Univariate Relationship Between DBP at Week 9 and Variables Measured at Week 5 (After 1 Month of Depletion) and Week 9 (After 1 Month of Repletion)

	Variables Measured at Week 5 (End of Depletion)			Variables Measured at Week 9 (End of Repletion)		
Variable	Correlation (r) With Week 9 DBP	P	Percent of DBP Variance Explained ($R^2\times100$)	Correlation (r) With Week 9 DBP	P	Percent of DBP Variance Explained ($R^2\times100$)
Ascorbate	−0.48	<0.0001*	23	−0.31	<0.01*	9
Beta-carotene	0.07	0.59	1	0.06	0.64	1
Alpha-carotene	−0.02	0.87	1	0.08	0.51	1
Cryptoxanthin	−0.05	0.71	1	0.11	0.38	1
Lutein	0.04	0.73	1	0.27	0.03	8
Lipid adjusted	−0.08	0.52	1	0.15	0.23	2
Lycopene	0.13	0.32	2	0.22	0.08	5
Lipid adjusted	−0.02	0.88	1	0.12	0.33	1
Selenium	−0.09	0.47	1	0.02	0.87	1
Retinol	0.11	0.38	1	0.12	0.32	2
α-Tocopherol	0.20	0.10	4	0.25	0.04	6
Lipid adjusted	0.07	0.59	1	0.08	0.54	1
γ-Tocopherol	0.14	0.27	2	0.12	0.36	1
Total cholesterol	0.22	0.08	5	0.26	0.03*	7
LDL cholesterol	0.19	0.12	4	0.25	0.04*	6

Sixty-eight men participated in a study of vitamin C depletion/repletion. During the study, participants did not smoke, drink alcohol, take any supplements, or consume any food other than that provided by the study. At the week 9 time point, participants had been on a controlled diet for 9 weeks and had undergone one full cycle of AscA depletion and repletion. Dietary factors, including depletion and repletion intakes of energy, calcium, sodium, potassium, and fiber, were also examined, and none was significantly associated with BP. *Significant predictor of BP.

trial, and 87% of both groups did so. Salonen et al[5] and Duffy et al[6] both found a statistically significant reduction in BP in subjects administered vitamin C compared with placebo.

Because of the research on oxidants and redox status in several BP-relevant biological systems and the preexisting literature on vitamin C and BP, we hypothesized that ascorbate status, change in ascorbate status, or both might be related to BP. The research presented here investigates whether vitamin C or other antioxidant status is associated with BP in a feeding study with a normotensive sample of subjects who did not smoke, drink, or alter their body weight during the 17-week study.

Methods

The National Cancer Institute (NCI), in collaboration with the US Department of Agriculture (USDA), undertook the present study to investigate several aspects of AscA metabolism. The study was approved by the Human Subjects Committees of NCI and USDA, and informed consent was obtained.

Study Design

Sixty-eight healthy men aged 30 to 59 years who had not smoked in the previous ≥6 months were recruited from the Beltsville, Maryland, area. Smokers were excluded so that the effects of dietary vitamin C intake could be examined unconfounded by any effect of smoking on BP and AscA. The subjects' mean age was 40.6 years (range 30 to 59 years), mean weight was 80.9 kg (range 59.3 to 100.8 kg), and mean body mass index (BMI) was 25.7 kg/m^2 (range 18.1 to 34.9 kg/m^2).

After 1 month of stabilization on 60 mg/d vitamin C intake, all participants underwent 1 month of vitamin C depletion (weeks 1 to 5) followed by 1 month of repletion (weeks 6 to 9). This pattern was then repeated: depletion (weeks 10 to 13) and then repletion (weeks 14 to 17). The design and methods have been reported elsewhere.[9,10] Subjects did not smoke, drink alcohol, use aspirin, take any supplements, or consume any food other than that provided in the study during the entire 17-week controlled-diet period. Energy intake was adjusted weekly to prevent weight gain or loss. Physical activity, stress, sickness, and other factors were recorded daily.

Diets

A 14-day rotating menu provided ≈50% of energy from carbohydrates and 36% from fat. The diet during the depletion periods contained 9 mg vitamin C/d provided by fruits and vegetables that contain little vitamin C: pear nectar, grape juice, apple juice, pears, fruit cocktail, applesauce, canned plums, raisins, pinto beans, iceberg lettuce, cucumber, celery, beets, corn, carrots, zucchini, mashed potatoes, mixed vegetables, and mushrooms. For repletion, subjects were randomized to receive 117 mg vitamin C from 1 of 3 sources: oranges, broccoli, or vitamin C supplement. The vitamin C supplement group continued to eat the fruits and vegetables in the depletion diet, as listed. The oranges and broccoli were assayed twice weekly, and amounts were adjusted to maintain identical dosages throughout the study.

During depletion, nutrient intakes averaged 3062 kcal, 992 mg calcium, 2498 mg potassium, 3291 mg sodium, and 22 g fiber. During repletion, the supplement group had a slightly lower intake of potassium (2450 versus 2664 mg in the fruit and vegetable groups) and dietary fiber (21.5 versus 23.4 g in the fruit and vegetable groups).

Compliance

Subjects were monitored during meals; uneaten food was negligible and had no effect on average vitamin C intake. Daily reports of

protocol violations and a final, postreimbursement anonymous questionnaire revealed that reported dietary omissions or commissions were minor and had no effect on average energy, vitamin C, or other nutrient intake. These monitoring procedures, similar to those of the DASH study, indicated excellent compliance with the dietary regimen.

BP Analyses

BP was measured with a Critikon Dinamap model 8100 Portable Blood Pressure Monitor (Critikon, Inc)[11] beginning in week 9, the end of the first repletion period. Subjects sat quietly for 5 minutes before the BP measurement, without talking, with the legs uncrossed, and with the arm supported at heart level. Measurement was repeated 3 times, and the average of the last 2 measures was used in the analyses.

Plasma Analyses

Fasting venous blood was collected biweekly during the depletion periods (weeks 3, 5, 11, and 13), and weekly during the repletion periods (weeks 6, 7, 8, 9, 14, 15, 16, and 17). Plasma was stabilized with 10% meta-phosphoric acid, and total ascorbate concentration was determined spectrophotometrically with 2,4-dinitrophenylhydrazine,[12] which correlates highly with HPLC methods.[13] AscA is presented here in units of mg/dL as well as in μmol/L, for comparison with earlier data. Plasma was also assayed for carotenoids, retinol, selenium, and α- and γ-tocopherol at the same weeks as plasma ascorbate and for cholesterol at weeks 1, 5, 9, 13, and 17.

Statistical Analyses

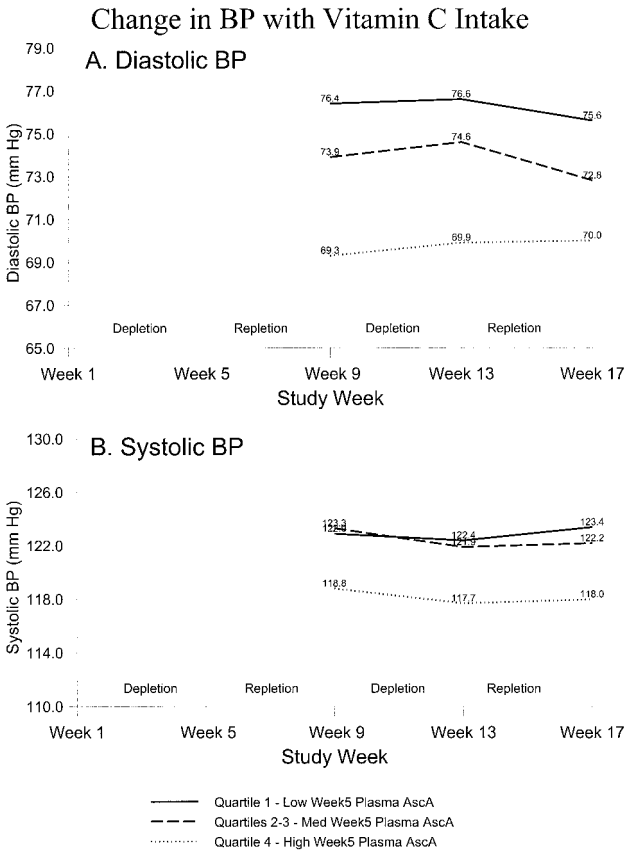
The main outcome in this study was not change in BP at the end of depletion and repletion, because subjects had already undergone 1 full depletion-repletion cycle before BP measurement was begun, and in both cases, they were incomplete depletions and repletions.[9] One third of the subjects had not reached a plateau in plasma AscA at the end of the first repletion, and half had not reached a plasma plateau at the end of the second repletion. Consequently, although changes in BP at various time points are presented here, the main outcome to be examined was the effect of baseline tissue stores (as reflected in plasma AscA retained after 1 month of depletion) on subsequent BP. Multiple regression, ANOVA, *t* tests, and Pearson's correlations were calculated with SAS Version 6.12, with significance at $P<0.05$.

Results

Subjects (57 whites, 8 blacks, and 3 Asians) had a mean diastolic BP (DBP) at week 9 of 73 mm Hg (range 53 to 97 mm Hg) and a mean systolic BP (SBP) of 122 mm Hg (range 103 to 143 mm Hg). Only 4 were hypertensive (DBP >90 mm Hg or SBP >140 mm Hg). Age, weight, and BMI were positively associated with BP and significantly so for age and DBP ($r=0.33$, $P=0.006$) (data not shown).

Average plasma ascorbate (AscA) during the last 2 weeks of depletion 1 (weeks 4 to 5) was 26.9 μmol/L (0.47 mg/dL), and during that period, >80% of subjects had an AscA level of >22.7 μmol/L, which is often considered the lower level of "normal."[14] At week 5, the end of depletion, AscA varied from 13.6 to 29 μmol/L (0.24 to 0.51 mg/dL), and one third of subjects were still above 22.7 μmol/L. On repletion, subjects attained a mean plasma AscA of 71.6 μmol/L (1.26 mg/dL) after the first repletion (week 9) and 60.2 μmol/L after the second repletion (week 17). The AscA attained on a given dose varied widely, and large numbers of subjects had not reached a plateau during either repletion period.[9]

Table 1 shows the relationship of week-9 DBP with several plasma variables measured at weeks 5 and 9. (None of the



A, Study design and DBP in weeks 9, 13, and 17 by quartiles defined by plasma AscA in week 5, age adjusted. Trend in DBP over AscA quartiles: week 9, $P=0.006$; week 13, $P=0.02$; week 17, $P=0.06$. B, Values for SBP.

variables shown in Table 1 were significantly associated with SBP in univariate analyses [data not shown]). Of the plasma nutrients examined, only ascorbate was significantly and inversely correlated with DBP ($r=-0.48$, $P<0.0001$ for week-5 AscA).

Table 1 suggests a stronger effect of the prospective association of AscA (week-5 AscA with week-9 DBP) than of the concurrent or cross-sectional association (week 9 with week 9). This is particularly notable because week-5 AscA, which is at the end of the first depletion period, may reflect tissue stores of AscA. It has been noted previously that persons who enter the depletion period with lower tissue stores have less reserves to preserve plasma levels, and thus plasma levels fall further.[9]

To initially explore this association of week-5 AscA with DBP, age-adjusted mean DBP over weeks 9, 13, and 17 was plotted by quartile of AscA at week 5 (Figure, A). Persons who had high AscA at week 5 had the lowest DBP at each time point (trend $P=0.007$ in week 9, $P=0.02$ in week 13, and $P=0.06$ in week 17). Overall, DBP rose 0.8 mm Hg on depletion ($P>0.1$) and fell 1.4 mm Hg on repletion ($P=0.1$). Those with high AscA at week 5 also had the lowest SBP at each time point (Figure, B), but these differences between groups and changes over time were nonsignificant for SBP.

Regression analyses were conducted to evaluate whether the apparent effect of week-5 AscA on DBP is due to other factors (Table 2). No other variable had a significant inverse association

TABLE 2. Effect of Other Variables on the Relationship Between DBP at Week 9 and Plasma Ascorbate

	Terms and Significance for Week-5 Ascorbate Variable		Terms and Significance for Added Variable		Percent of Variance Explained by Model ($R^2\times100$)
Added Variable	Partial F*	P	Partial F*	P	
Only Week-5 AscA	18.56	<0.0001	...	...	23
Age	13.88	<0.0001	3.98	0.05	27
Weight	16.17	<0.0001	0.27	0.61	23
BMI	16.42	<0.0001	0.63	0.43	24
Diet in Weeks 1–5					
Energy, Kcal	18.35	<0.0001	0.09	0.77	23
Calcium, mg	18.37	<0.0001	0.10	0.75	23
Fiber, g	18.45	<0.0001	0.25	0.62	23
Sodium, mg	18.41	<0.0001	0.12	0.73	23
Potassium, mg	18.36	<0.0001	0.11	0.74	23
Diet in Weeks 6–9					
Energy, Kcal	18.51	<0.0001	0.19	0.66	23
Calcium, mg	18.38	<0.0001	0.33	0.56	23
Fiber, g	21.30	<0.0001	4.64	0.04	28
Sodium, mg	19.14	<0.0001	0.93	0.34	24
Potassium, mg	21.74	<0.0001	5.52	0.02	29
Took stairs on BP day	23.23	<0.0001	6.54	0.01	30
Beta-carotene	18.91	<0.0001	0.73	0.40	24
Alpha-carotene	19.35	<0.0001	1.39	0.24	25
Lutein	15.02	0.0003	2.38	0.13	26
Lycopene	18.05	<0.0001	3.08	0.08	27
Selenium	18.40	<0.0001	0.03	0.87	23
α-Tocopherol	15.32	<0.0001	1.83	0.08	25
Lipid adjusted	17.87	<0.0001	0.07	0.79	23
γ-Tocopherol	17.50	<0.0001	0.25	0.62	23
Plasma cholesterol	15.48	0.0002	2.31	0.13	26
Hematocrit	13.68	0.0005	3.19	0.08	27
Treatment group	19.78	<0.0001	1.83	0.12	33

All models are of the form DBP=week-5 ascorbate+added variable.

*The partial F test assesses whether the addition of that variable contributes significantly to the prediction of DBP.

with DBP. The week-5 AscA level remained highly significant ($P<0.001$) even after control for any of the plasma or dietary variables shown. All 3 repletion source groups (vitamin C supplement, oranges, or broccoli) had similar week-9 DBPs (73.6, 72.3, and 74.2 mm Hg, respectively); the source of vitamin C was not itself significant in ANOVA ($P=0.89$) and did not alter the significance of the ascorbate effect. Sickness, injury, and days of vigorous exercise did not alter the association between AscA and DBP (data not shown).

In final multiple regression models, AscA quartile at week 5 was the most significant factor associated with week-9 DBP (Table 3). Each increase in week-5 AscA quartile was associated with 2.4 mm Hg lower DBP at week 9 ($P=0.003$). The week-5 AscA quartile was still negatively associated with DBP at week 13 ($P=0.02$) but was no longer significantly associated with DBP at week 17 ($P=0.09$). AscA was inversely but not significantly associated with SBP at weeks 9, 13, and 17 (data not shown).

For ease in examining the association between AscA and BP at their conventional cutpoints of normal (or optimal in the case of BP),[14,15] subjects were divided on the basis of week-9 BP (> or <DBP 80 mm Hg or SBP 120 mm Hg) and on the basis of AscA (week-5 AscA > or <22.7 μmol/L AscA [0.4 mg/dL]) (Table 4). In this prospective analysis (week-5 AscA, week-9 DBP), only 1 out of 24 persons (4%) who had maintained ≥22.7 μmol/L AscA throughout weeks 1 to 5 had week-9 DBP of >80 mm Hg, compared with 11 out of 44 (25%) of those with lower AscA. The relative risk (RR) was 6.0, and adjustment for age, BMI, and dietary variables increased the RR to 7.2. There also was a statistically significant effect for SBP, RR of 1.9 unadjusted, which increased to RR of 4.0 after adjustment. Examined cross-sectionally (week-9 AscA, week-9 DBP), none whose week-9 plasma AscA values reached 79.5 μmol/L (sometimes considered the upper limit of "normal") had DBP of >80 mm Hg, whereas about one third of the men with lower plasma AscA values had this level of DBP (data not shown).

TABLE 3. Multiple Regression Analysis of DBP, Ascorbate Status, and Other Variables: AscA as Week-5 Quartiles

	Change in DBP, per unit change in variable	Partial *F*	*P*	Model R^2
Week-9 DBP				0.35
Plasma ascorbate at end of first depletion (week 5) (quartiles 1–4)	−2.40	9.6	0.003	
Age, y	+0.25	4.9	0.03	
BMI, BMI units	+0.09	0.1	0.76	
Energy, 100 Kcal	−3.29	3.6	0.06	
Sodium, 100 mg	+4.28	3.5	0.07	
Fiber, g	+0.02	0.0	0.95	
Week-13 DBP				0.35
Plasma ascorbate at end of first depletion (week 5) (quartiles 1–4)	−2.20	5.9	0.02	
Age, y	+0.37	8.1	0.01	
BMI, BMI units	+0.10	0.1	0.77	
Energy, 100 Kcal	−4.95	5.9	0.02	
Sodium, 100 mg	+6.54	5.9	0.02	
Fiber, g	−0.27	0.5	0.49	
Week-17 DBP				0.27
Plasma ascorbate at end of first depletion (week 5) (quartiles 1–4)	−1.63	2.9	0.09	
Age, y	+0.37	7.3	0.01	
BMI, BMI units	+0.49	0.8	0.34	
Energy, 100 Kcal	−1.91	0.8	0.38	
Sodium, 100 mg	+2.66	0.9	0.35	
Fiber, g	−0.24	0.4	0.55	

All other variables mentioned in the text, as well as stress, sick days, physical activity days, passive smoke exposure, serum carotenoids, and others, were examined, were not statistically significant, and did not alter the AscA variable. Dietary variables used in these analyses represent the nutrients in the diets provided by the study in the 4 weeks that preceded each BP measurement.

Mean μmol/L (mg/dL) AscA in each week-5 quartile: 17.2 (0.3), 20.4 (0.4), 22.2 (0.4), and 25.2 (0.5).

Regression coefficient for the relationship between week-9 (repleted) AscA quartile and week-9 DBP was −1.2. Mean μmol/L (mg/dL) AscA in each week-9 quartile: 55.9 (1.0), 68.3 (1.2), 75.8 (1.3), and 86.6 (1.5).

Discussion

This study provides evidence that plasma ascorbate is inversely associated with BP, even in healthy subjects with a quite low range of BP. Both after several weeks at blood AscA levels lower than most Americans and at blood levels higher than most Americans, an association with BP was found for AscA and not for other plasma nutrients.

A number of design aspects represent improvements over previous studies. Habituation to an intervention protocol, and regression to the mean, often results in declines in BP for those reasons alone, even on placebo treatment.[16] This study, which did not select subjects on the basis of elevated BP and which did not begin BP measurement until week 9, avoids those potential artifactual results, and BP changes between week 9 and week 13 are in the opposite direction as what would be expected from habituation. Nutrient intake was controlled, avoiding potential confounding by changes in subjects' intake of dietary fat, potassium, or calcium. In addition, nutrients such as potassium and fiber were controlled in multivariate models and did not alter the AscA effect. Body weight was monitored weekly, and energy intake was adjusted to prevent changes; thus, changes in body weight could not explain the BP effects. Subjects did not smoke or consume alcohol, behaviors associated with alterations in vitamin C status and BP. A number of plasma nutrients were evaluated in addition to ascorbate, and detailed data on subjects' illnesses, exercise habits, stress, passive smoke exposure, and other factors were also examined. Finally, the prospective nature of the results and the very low BP of the subjects make it unlikely that hypertension caused low AscA.

There also were study limitations. It is possible that the AscA effect was due to an unmeasured correlate of AscA metabolism. However, numerous potential confounders were evaluated, as noted earlier, and none was found to alter the AscA effect. Other plasma nutrients were only weakly correlated with AscA ($r<0.2$).

Nor is this an adequate study of change in BP, despite its depletion/repletion design. The initial changes of interest, between the first depletion and the first repletion, could not be

TABLE 4. Relationship Between Plasma Ascorbate at Week 5 and BP at Week 9, With Both Variables Dichotomized at Their Standard Definitions of Normal

	Week-9 DBP		Week-9 SBP	
	Above 80 mm Hg	Below 80 mm Hg	Above 120 mm Hg	Below 120 mm Hg
Week-5 AscA				
<22.7 μmol/L (0.4 mg/dL)	11	33	28	16
≥22.7 μmol/L (0.4 mg/dL)	1	23	8	16
Unadjusted				
Relative risk[1]	RR=6.0		RR=1.9	
Fisher's exact test of association	*P*=0.03		*P*=0.02	
Adjusted[2]				
Relative risk[1]	RR=7.3		RR=4.0	
Wald *P*	*P*=0.08		*P*=0.02	

[1]Risk of having BP above normal if subject was below 22.7 μmol/L AscA.
[2]In logistic regression analysis with adjustment for age, BMI, energy, sodium, and fiber. None of the variables other than AscA approached statistical significance.

examined because BP measurement was not begun until the first repletion had been completed. Furthermore, an adequate study of change would require a sufficiently long intervention to permit tissue stores to reach a fully repleted steady state. The absence of plateaus in plasma levels in these subjects[9] provides evidence that body pools and tissue stores had not reached a steady state after the depletion and repletion end points. These factors make interpretation of the changes with depletion and repletion difficult.

Nevertheless, the magnitude of BP changes is consistent with those of the DASH study for nonhypertensives. The fall in DBP in the present study between week 13 (depletion) and week 17 (repletion) (on average, 1.4 mm Hg) is greater than the 0.3 mm Hg seen in the DASH fruit/vegetable group and well within the confidence interval of the DASH study combination group (2.1 mm Hg, CI 0.5 to 3.6).

The depletion and repletion conditions in this study are relevant to conditions in developed countries. The repletion intake of vitamin C, 117 mg/d, is easily achievable in a normal diet and is approximately the average amount consumed by most Americans.[17] The blood AscA levels achieved on repletion are seen in US data; among men in this age range in the Second National Health and Nutrition Examination Survey (NHANES II),[17] the 75th percentile was ≈73.8 μmol/L (1.3 mg/dL), comparable to the 75th percentile of 79.9 μmol/L at week 9 in the present study. The depletion levels are also relevant to the US data. Although the vitamin C fed during depletion is lower than the intake of most Americans, the blood AscA levels experienced during depletion are found in a substantial proportion of the US population. The average AscA during the last 2 weeks of depletion was 26.9 μmol/L; in NHANES II, ≈20% of American men in this age range had blood AscA levels this low or lower. An even higher proportion of black men, ≈30%, had blood AscA levels in this range.[17]

Blacks have higher rates of hypertension and associated disabilities[18] and lower levels of AscA as noted earlier. This study suggests that some of the hypertension among blacks may be due to low AscA levels, an observation consistent with other data.[19]

The cross-sectional association between AscA and DBP ($r=-0.31$) was as strong as the age-DBP association ($r=0.33$) in this study (Tables 1 and 2) and similar to cross-sectional correlations between AscA and BP in earlier studies. However, the data presented here represent an advance over cross-sectional analyses for several reasons: (1) the study provided all of the food consumed by subjects, and therefore other dietary factors could be and were examined; (2) the AscA association was seen at all time points and to the exclusion of other plasma and dietary nutrients; and (3) the study design permitted examination of an AscA-depleted state that may reflect tissue stores. It is this latter feature that suggests a role for AscA and suggests possible mechanisms.

The level of plasma AscA retained after a period of low intake indirectly reflects long-term tissue stores before the study. Subjects who enter the week-1 to -5 depletion period without full tissue AscA saturation have lower initial tissue stores to maintain plasma levels during depletion, resulting in lower plasma AscA levels at week 5. It was AscA levels at this point that were most strongly associated with BP ($r=-0.48$ in Table 1). Thus, these results suggest that it may be total body AscA stores, or AscA concentrations in BP-relevant tissues, that are important in preventing elevated BP.

Pharmacokinetic studies have indicated the presence of ≥3 ascorbate "compartments," of which plasma is only 1.[20,21] Furthermore, numerous human organs and tissues import AscA against a concentration gradient, achieving very high levels. Adrenal and pituitary glands have ≈100 times the plasma concentration of AscA, and liver, spleen, pancreas, brain, and eye lens have ≈20- to 30-fold the plasma concentration, in both rats, guinea pigs, and humans.[21] In chronic marginal intake, certain key organs may have higher priority than vascular cells or other organs related to BP, resulting in chronic undersupply in relation to their optimal requirements.

This hypothesis could explain why week-5 AscA was the strongest predictor of subsequent BP: persons who reached lower levels at week 5 had lower total body stores, possibly indicating chronic undersupply in the vascular or other BP-relevant tissues, and those tissues were incompletely repleted in

subsequent cycles. Thus, the value of the week-5 data is not in suggesting that deficient vitamin C intake is associated with elevated BP (>80% of subjects had "normal" AscA during the last 2 weeks of depletion) but in suggesting that *body stores* of AscA may be associated with BP.

This hypothesis of vascular tissue–specific AscA depletion is consistent with a number of biological mechanisms that could explain an AscA-BP relationship; these include prevention by AscA of free radical inhibition of prostacyclin synthetase, prevention by AscA of nitric oxide inhibition of release of endothelium-derived relaxing factor,[22] promotion by AscA of endothelial prostacyclin production, effects of AscA on smooth muscle contractility of peripheral blood vessels and improvement of vasomotor dysfunction, effects of AscA on aortic collagen, and others.[23–27]

MacMahon et al[28] estimated that a reduction of 2 mm Hg in average population DBP could be associated with 14% fewer strokes and 8% less coronary heart disease. Table 3 indicates a potential effect of 2.4 mm Hg DBP for each higher quartile of AscA, over the range of AscA values seen at week 5. Over the range of AscA values attained at week 9 after 1 month on 117 mg/d, a potential effect of 1.2 mm Hg for each higher week-9 AscA quartile is indicated. The AscA blood levels seen in this study, at both the lower and the upper ends, are well within the levels seen routinely in the US population. These data suggest the possibility of important reductions in BP if adequate AscA tissue stores are achieved and maintained. Because this study was not a randomized placebo-controlled trial, it cannot definitively address the question of whether normal levels of DBP could be reduced even further if higher blood levels of ascorbate were attained and maintained (nor whether hypertension could be reduced, because subjects were normotensive). Long-term, well-designed interventions are clearly needed, but this study provides substantial evidence for a possible role for AscA in the prevention of elevated BP.

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